AD-A090 183 BIOMEDICAL RESEARCH INST ROCKVILLE MD F/G 6/5
CRYOPRESERVATION OF SCHISTOSOME LARVAE.(U)
OCT 80 M A STIREWALT, C S RICHARDS N00014-78-C-0081
UNCLASSIFIED NL

AD A090183

UNCLASSIFIED

A074564

12

(15) OFFICE OF NAVAL RESEARCH
Contract No. N00014-78-C-0081
Task No. NR 204-072
(9) Annual Report No. 3

(6) Cryopreservation of Schistosome Larvae

by

(10) M. A. Stirewalt C. S. Richards
J. T. Sullivan

Biomedical Research Institute
12111 Parklawn Drive
Rockville, Maryland 20852

OCT 7 1980
D
C

(11) 2 October 1980
(12) 76

Reproduction in whole or in part is permitted for any purpose of the United States Government.

This document has been approved for public release; its distribution is unlimited.

DDC FILE COPY

388117

80 10 7 060

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: 3	2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE *(and Subtitle)*: Cryopreservation of Schistosome Larvae	5. TYPE OF REPORT & PERIOD COVERED: ANNUAL REPORT 30 Sept. 1979-1 Oct 1980
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): M. A. Stirewalt, C. S. Richards & J. T. Sullivan	8. CONTRACT OR GRANT NUMBER(s): N00014-78-C-0081
9. PERFORMING ORGANIZATION NAME AND ADDRESS: American Foundation for Biological Research, 12111 Parklawn Drive, Rockville MD 20852	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: NR 204-072
11. CONTROLLING OFFICE NAME AND ADDRESS: Procurement Contract Officer, Office of Naval Research (433) Dept. of the Navy, Washington, D. C. 20360	12. REPORT DATE: 2 October 1980
	13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)*	15. SECURITY CLASS. *(of this report)*: Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Standard Distribution List

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Schistosoma mansoni; trematode: cryopreservation; vaccine; irradiated cercariae; protective immunity; genetic variations of S. mansoni; Isozymes of S. mansoni; Biomphalaria glabrata; susceptibility of B. glabrata; genetics of B. glabrata; amebocytes, molluscan; encpasulation; molluscicides

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Seventeen genetically different strains of S. mansoni and sixteen stocks of B. glabrata showing genetic differences in susceptibility patterns are being composed. Isozyme studies on 13 S. mansoni strains and 5 B. glabrata stocks demonstrated differences in all. Histologic studies on B. glabrata stocks exposed to S. mansoni demonstrated three types of host reaction: active resistance, passive unsuitability, and susceptibility. EM studies on one stock of B. glabrata showed amebocytes attacking and destroying muscle cells of the atrium, a reaction against self. Molluscicidal effect of cashew nut shell extract for B. glabrata was shown to be due primarily to the triene component of anacardic acid.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE S/N 0102-LF-014-6601

80 10 16 078

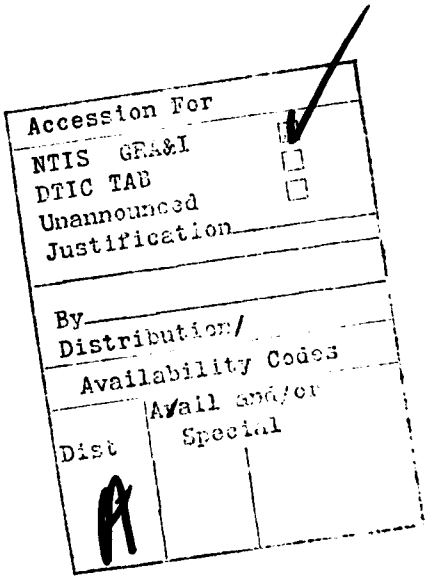
SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

Background

Significant protective immunity to Schistosoma mansoni develops in several mouse strains (a) during a patent chronic infection, and (b) following immunization with highly irradiated viable cercariae. Since injection of highly irradiated cercariae does not lead to a patent schistosomal infection this type of immunization shows potential for human vaccine use.

Antigenic polymorphism within a single isolate of Schistosoma mansoni appears to affect the resistance of mice to second infection with various clones developed from the same isolate (Smith and Clegg, Parasitology 78: 311-321, 1979). Such antigenic polymorphism has important implications for experimental studies of immunity to schistosomiasis and also for the development of a vaccine.

Strains of S. mansoni, including isolates from different geographic areas, multiple isolates from some areas, and multiple substrains selected from individual isolates have been differentiated on the basis of infectivity for intermediate host snails. This series of genetically different S. mansoni strains provides parasites for studies on variations in immunologic and pathologic aspects of different S. mansoni strains, and differences in cryopreservation qualities as means of storing them for vaccine production.



Methodology and Results

B. glabrata snails have been exposed, both as juveniles and adults, to a variety of strains of S. mansoni. By selection on the basis of susceptibility and isolation of snails as juveniles with reproduction by self-fertilization only, a series of inbred snail lines has been established showing different patterns of susceptibility. Exposures of snails from these characterized lines to miracidia of S. mansoni serves to differentiate genetic infectivity patterns in different parasite strains.

(1) Intraspecific genetic variations in susceptibility for S. mansoni strains were compared in 16 stocks of B. glabrata. About 10 of these snail stocks are routinely used in testing variations in S. mansoni infectivity. Isozyme studies on 5 of the B. glabrata stocks demonstrated differences. Snail crosses, involving 16 stocks of B. glabrata, have been made in studies on inheritance of genetic factors influencing susceptibility for S. mansoni infection. Some of these crosses, and analyses of the results, are still in progress.

(2) Studies on intraspecific genetic variations in S. mansoni have been continued. Seventeen strains have been maintained and compared, including: 13 of Puerto Rican origin, 3 of St. Lucian origin, and on Egyptian. Differences in isozymes (Fletcher et al., submitted) and in snail infectivity patterns were demostrated in these S. mansoni strains. Isozyme variations for one enzyme system, lactate dehydrogenase (LDH), appear to be associated with variations in infectivity for B. glabrata. Development of inbred lines of the S. mansoni strains, by brother-sister matings, are in progress. Crosses have so far been performed involving six of the S. mansoni strains, to study inheritance of snail infectivity factors.

(3) Cercariae of several different strains of S. mansoni have been provided to NMRI and to LPD, NIAID, NIH for immunologic studies.

(4) Histologic studies on several different stocks of B. glabrata exposed to one strain (NIH-Sm-PR2) of S. mansoni helped clarify variations in host-parasite interactions (Sullivan and Richards, submitted). Snail host reactions are of three types (with some intergradations): (1) resistance (active encapsulation and destruction of parasites by amebocytes); (2) unsuitability (failture of parasite development with minimal or no host reaction); and (3) susceptibility (normal parasite development with no host reaction). Snail crosses indicated that resistance is dominant over unsuitability and susceptibility, and unsuitability, dominant over susceptibility.

(5) EM studies demonstrated that genetically regulated amebocytic accumulations in in the atrium in one stock of B. glabrata produced increased amounts of lysosomal enzymes and attacked and destroyed muscle cells of the atrium, a phenomenon resembling vertebrate autoimmunity (LoVerde et al., submitted).

(6) Among 7 genetic stocks of B. glabrata nonsusceptible to the NIH-Sm-PR2 strain of S. mansoni, 5 stocks reverted to susceptibility, one to partial susceptibility, and one remained nonsusceptible, when first infected with Echinostoma paraensei (Sullivan et al., submitted).

(7) B. glabrata sensitized by infection with x-irradiated miracidia of Ribeiroa marini acquire a strong resistance to a challenge infection with normal R. marini miracidia (Sullivan et al., submitted).

(8) Tolerance to four molluscicides was compared in a laboratory stock and a field stock of B. glabrata (Sullivan and Richards, submitted). The laboratory snail stock demonstrated more resistance for PCP, Bayluscide, and $CuSO_4$. The field stock was slightly more resistant to Frescon.

(9) The saturated monene, diene, and triene components of anacardic acid, obtained by fractionation of an extract from the cashew nut shell, were tested for toxicity to B. glabrata. The triene form was most toxic, the di—and monene forms less toxic, and the saturated form relatively nontoxic. Results suggested that definitive laboratory testing of this naturally-occuring molluscicide is justified (Sullivan et al., submitted).

Discussion

Six isolates of S. mansoni from three geographic areas, have yielded 17 parasite strains by selection on the basis of infectivity for B. glabrata snails. These parasite strains showed genetic variations in infectivity patterns. Isozyme studies on 13 of the strains demonstrated enzyme differences. The results indicated the need for further genetic characterization of parasite strains used in biomedical research, and comparative studies on the influence of strain differences on immunology, pathology, epidmiology, etc. Strains from additional geographic areas should be tested, characterized, and compared.

Collaborative studies at Purdue University (Fletcher and LoVerde) suggested that isozyme variations for at least one enzyme system, lactatedehydrogenase, in our S. mansoni strains were associated with variations in snail infectivity. Such studies should be continued.

Studies on the histology of the snail-parasite interaction, comparing a series of stocks of B. glabrata exposed to S. mansoni strain NIH-Sm-PR2, demonstrated that the host relation was not simply nonsusceptible or susceptible. Nonsusceptible stocks could be actively resistant, passively unsuitable, or cominations of these phenomena. These studies should be extended to compare results with different parasite strains, and the genetics of these variations in both host and parasite explored further.

Snail exposures and selection resulted in establishing 13 strains of S. mansoni from 5 isolates of Puerto Rican origin. These strains differed genetically in patterns of infectivity for B. glabrata. Electrophoretic studies on 11 of the strains demonstrated izozyme differences. Four samples of B. glabrata from different localities in Puerto Rico are demonstrating differences in susceptibility for the Puerto Rican S. mansoni strains.

Crosses are in progress between snails of different stocks and parasites of different strains. The studies provide some information on the epidemiology of schistosomiasis, but more snail samples and parasite isolates should be obtained and compared.

Accomplishments during this report period include:

Significant Accomplishments

(1) A wide range of intraspecific genetic variations has been demonstrated in both S. mansoni and B. glabrata: 17 strains of S. mansoni showing different patterns of snail infectivity, and 16 stocks of B. glabrata showing different patterns of parasite susceptibility are under study;

(2) Isozyme differences were demonstrated in 13 strains of S. mansoni and 5 stocks of B. glabrata studied;

(3) Histologic studies on a series of stocks of B. glabrata exposed to one strain of S. mansoni showed the host reaction could be active resistance, passive unsuitability, or susceptibility. Resistance is genetically dominant over unsuitability and susceptibility; unsuitability, dominant over susceptibility;

(4) EM studies showed that in one stock of B. glabrata, with genetic accumulations of amebocytes in the atrium, the amebocyties attacked and destroyed muscle cells of the atrium, a reaction against self; and

(5) Molluscicidal effect of cashew nut shell extract for B. glabrata was shown to be due primarily to the triene component of anacardic acid.

BIBLIOGRAPHY

1. Lie, K. J., Heyneman, D., and Richards, C. S.: Specificity of natural resistance to trematode infections in Biomphalaria glabrata. Internat. J. Parasit., 9: 529-531, 1979.

2. Richards, C. S.: Genetic studies on amebocytic accumulations in Biomphalaria glabrata. J. Invert. Pathol., 35: 49-52, 1980.

3. Richards, C. S.: Inherited abnormal growths on the mantle of Biomphalaria glabrata. J. Invert. Pathol., 35: 119-123, 1980.

4. Richards, C. S.: Abnormal growths from the digestive gland of Biomphalaria glabrata. J. Invert. Pathol., 35: 318-319, 1980.

5. Richards, C. S.; Edema-horn, an abnormal mutant of Biomphalaria glabrata. J. Invert. Pathol., 35: 53-57, 1980.

6. Richards, C. S.: Abnormal development of the digestive gland in Biomphalaria glabrata. J. Invert. Pahtol., 36: 121-122, 1980.

7. Fletcher, M. LoVerde, P. T., and Richards, C. S.: Electrophoretic characterization of strains of Schistosoma mansoni from Puerto Rico and St. Lucia selected for high or low infecitivity to the intermediate host. Exp. Parasit. (submitted, 1980).

8. LoVerde, P. T., Gherson, J., and Richards, C. S.: Reaction against self in an invertebrate? J. Invert. Pathol., (submitted, 1980).

9. Kasschau, M. R. and Richards, C. S.: Concentration changes in free amino acid pools during the life cycle of Schistosoma mansoni. (submitted, 1980).

10. Sullivan, J. T., Richards, C. S., Lie, K. J., and Heyneman, d.,: Ribeiroia marini: acquired resistance in Biomphalaria glabrata induced by irradiate miracidia. Exp. Parasit. (submitted, 1980).

11. Sullivan, J. T., Richards, C. S. Lie, K. J., and Heyneman, D.,: Protection of Schistosoma mansoni, NIH-SM-PR-2 strain, in nonsusceptible Biomphalaria glabrata by Echinostoma paraensei, Internat. J. Parasit., (submitted, 1980).

12. Sullivan, J. T., & Richards, C. S.: Schistosoma mansoni, NIH-SM-PR-2 strain in susceptible and nonsusceptible stocks of Biomphalaria glabrata: Comparative histology. Jour. Parasit. (submitted, 1980).

13. Sullivan, J. T. & Richards, C. S.: Increased susceptibility to infection with Ribeiroia marini in Biomphalaria glabrata exposed to X-Rays. (submitted, 1980).

14. Sullivan, J. T. & Richards, C. S.: Comparative mortality of a laboratory and field stock of Biomphalria glabrata exposed to four molluscicides. Bull. WHO, (submitted, 1980).

15. Sullivan, J. T., Krishna, G., Lloyd, H. A., & Richards, C. S.: Molluscicidal activity of anacardic acid. Bull. WHO, (submitted, 1980).


LMED